IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Docket No. 30307A
KNOLL, JOAN et al.	
Serial No. : 09/854,867	Group Art Unit No. 1634
Filed: May 14, 2001	
SINGLE COPY GENOMIC HYBRIDIZATION PROBES AND METHOD OF GENERATING THE SAME	Examiner: C. Myers

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

DECLARATION

WE, JOAN H. M. KNOLL and PETER K. ROGAN, declare and state as follows:

1. We are the inventors of the subject matter described and claimed in the above-identified patent application. Attached are curriculum *vitaes* for each of the inventors.

2. This declaration is being submitted by us pursuant to 37 C.F.R. § 1.132 in order to submit evidence pertaining to the prior art and state of the art at the time the invention was made brought to attention in the office action dated August 5, 2004 and the interview that took place on December 7, 2004.

3. The Office Action included an allegation that the sequences of Tanaka et al., 113 Cancer Genetics and Cytogenetics, 29-35 (1999) anticipated claims 44 and 45 of the present application. Prior to this amendment, the claims recited "single copy" but this phrase has been replaced by the limitation that the probes are free of SEQ ID Nos. 1-428 and 447-479. Under either limitation, it cannot be said that Tanaka anticipates the claims. This is because Tanaka

contains repetitive sequences. Specifically, the AML1 gene is shown by way of a restriction map which shows that the probes disclosed therein contain both introns and exons. Figure A, attached hereto, shows the distribution of repetitive sequences in the AML1 gene. These repetitive sequences are included in SEQ ID Nos 1-428 and 447-479.

4. The present application also demonstrates that the probes do not require prehybridization or cohybridization with unlabeled repetitive DNA sequences to provide hybridization specificity. The methods and probes of Tanaka require such prehybridization or cohybridization using C_ot-1 DNA in order to prevent cross hybridization with the repetitive sequences contained in the probes. Accordingly, in order to provide hybridization specificity, Tanaka uses C_ot-1 DNA to mask or block this non-specific hybridization. Such is not required by the present invention as the probes therein contain no repetitive sequences that must be blocked or masked.

5. During the interview, a question regarding the prior art was asked. This question was essentially this: At the time the invention was made, were there any other probes or methods of using probes, that were developed using the methods of the present invention, that were single-copy as defined in the application? The answer to this question is no. We were the first ones to systematically and thoroughly screen for single copy probes. This was accomplished by 1) comparing the nucleotide by nucleotide sequence of nucleic acid sequences with a host of repetitive sequences; 2) using the intervals between the identified repetitive sequences as probes for detecting nucleic acid abnormalities; and 3) using the intervals between the identified repetitive sequences as probes for localizing the interval in which such abnormalities occur. The present claims require that the probes to be free of SEQ ID Nos 1-428

and 447-479 and because these sequences are the repetitive sequences, any probe that is free of these sequences is covered by our definition of single copy that is contained in the application. To the best of our knowledge, prior art methods required prehybridizing or cohybridizing the genomic probes with unlabeled repetitive DNA sequences, such as C_ot-1 DNA, to provide hybridization specificity. We further note that the present claims also require that the probes be compared with the above-referenced repetitive sequences as part of the claimed method. We were also the first people to do such a comparison in the development of our probes.

6. In addition to our assertion of being the first to invent probes using the claimed methods, several other lines of evidence support such an assertion. Repeat sequence databases, such as those encompassed by SEQ ID Nos: 1-427 and 447-479, were previously used to identify and characterize other repetitive sequences in genomic DNA and to catalog the types of repetitive sequences. These applications for probes are not directly related to the utility of the present invention and there is no indication within those applications of the methods described and claimed in the present application, nor of the utility of such methods. Thus, at the time of invention, our invention was not obvious to those developing the repeat sequence databases. Furthermore, even if it were conceived of by others, it would be essential to prove to those of skill in the art that the database of repetitive sequences was sufficiently comprehensive as to identify the single copy nature of sequences from a sufficient number of genomic loci. We have performed this additional step as evidenced in the present application, thereby providing sufficient proof of utility to those of skill in the art. This reduction to practice cannot be carried out computationally (see the references below) and must be verified in the laboratory for the invention to have specific, credible and convincing utility to those of skill in the art. To the best

of our knowledge, computational approaches to searching for single copy sequences are generally comprehensive and are an important step in verifying single copy nature of potential probes but those of skill in the art would not consider them to be sufficient evidence of the validity of the instant invention. However, despite common belief, the reference genome sequence is not complete since the short arms of acrocentric chromosomes have not yet been sequenced. Also, the reference sequence is a composite of the sequences from multiple individuals. It has become clear in the past 6 months that copy number polymorphisms are present at 5-6% of the human genomic loci, hence the reference sequence is a compromise that is useful for searching for single copy sequences, but it will not be correct for all individuals. Copy number polymorphisims may be manifested as duplicons or triplicons, according to the probes of the instant invention.

We reduced the concept of single copy probes defined in the instant invention to practice using the best mode (ie the most stringent criteria) available to prove that these probes were truly single copy. Molecular cytogenetic techniques such as FISH, Southern hybridization analysis, certain quantitative PCR techniques can directly demonstrate that the probes produced by the invention are single copy. Others (eg. Rinn et al. Genes Dev. 2003 Feb 15;17(4):529-40) currently practicing our invention have not performed rigorous testing of the probes to demonstrate their single copy nature. In fact, they validated probes with the same indirect approach that Croce and Tanaka used to identify single copy sequences (by their failure to hybridize to repetitive sequences in whole genome-labeled DNA). We have previously shown, in the prosecution of the '097 patent and in this declaration, that this technique is not as sensitive or specific as the molecular cytogenetic approach we used and are claiming herein. As a result, it underestimates the repetitive sequence content of presumably single copy probes, rendering

them less useful. Evidence for this is the fact that despite their claims of "single copy" probes, their sequences have contained at least on of SEQ ID Nos 1-427 and 447-479.

Furthermore, the method of the present invention is enabling to those of skill in the art, since we completely specified the repetitive sequence library used to develop these probes. Others currently practicing our invention do not specify the repetitive sequence library and refer only to the software that compares genomic sequences within which probes were developed with repetitive sequences (Rinn et al. 2003; Buckley et al. Hum Mol Genet. 2002 Dec 1;11(25):3221-9). The software itself does not teach how to make such single copy probes, it simply does the comparisons between the repeat database and the genomic sequence.

7. In further support of the assertion that we were the first to use probes developed using the methods recited in the claims, we note that we were recently issued U.S. Patent No. 6,828,097. Accordingly, neither a search by the U.S. Patent Office nor the searches undertaken by the inventors, found any probes made as claimed therein. The claims that issued in this patent include similar limitations that are included herein. For example, claim 1 of the issued patent recites "determining the sequence of at least one single copy sequence in said target nucleic acid sequence computationally, said determining step comprising the steps of ascertaining the nucleotide-by-nucleotide sequence of said target nucleic acid sequence, comparing said ascertained sequences with the sequences of SEQ ID Nos. 1-428 and 447-479 in said target nucleic acid sequence using a computer program, and identifying said single copy sequence from said comparison." The independent claims of the the present application recite similar methods for identifying the probe sequences in that all of the independent claims require comparing nucleic acid sequences with SEQ ID Nos 1-428 and 447-479 and selecting sequences that are free of those SEQ ID Nos. for use as probes.

8. We further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further that those statements were made with the knowledge that wilful, false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and such wilful false statements may jeopardize the validity of any patents issued from the patent application.

Date: Dec. 22, 2004

PETER ROGAN

Date: Dec. 22, 2004

JOAN KNOLL